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# A Cross-Sectional Study of Levels of Total Antioxidants, Vitamin D, Ferritin, and Glycated Hemoglobin in Males with Type 2 Diabetes Mellitus

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**Abstract.** Diabetes, a long-term condition linked with substantial morbidity, increased mortality, and escalating healthcare expenses, is quickly emerging as a worldwide epidemic. The present investigation aims to evaluate ferritin levels, glycated hemoglobin, total antioxidant status and serum vitamin D concentrations within a population of diabetic men and to identify any correlations that may exist between these parameters. This study was conducted from 2023 to 2024 and involved 50 patients with T2DM and 50 healthy controls aged 40-65. Results show an increase levels of vitamin D, ferritin, and HbA1c in patients compared to healthy controls. While total antioxidant show decrease levels in patients compared to healthy controls. The significant role of increased ferritin in pathogenesis of diabetes mellitus type2 due to accumulation of ferritin in the liver prevents insulin from responding. In Iraqi diabetic patients, there is a strong link between circulating ferritin levels and total antioxidants, while vitamin D status could contribute to maintaining glucose homeostasis.

**Keywords:** Diabetes mellitus, ferritin, glycated hemoglobin, total antioxidant, Vitamin D

## INTRODUCTION

Diabetes, a long-term condition linked with substantial morbidity, increased mortality, and escalating healthcare expenses, is quickly emerging as a worldwide epidemic. The worldwide prevalence of diabetes is expected to rise from 171 million in 2000 to 366 million by the year 2030 [1]. Recent Data Research from both animal and clinical studies involving humans indicates that vitamin D may help reduce the risks linked with diabetes [2]. Diabetes Mellitus is a chronic metabolic disorder distinguished by high bloodstream glucose amounts (hyperglycemic) secretion, impaired insulin function, or a combination of both. It is one of the most prevalent diseases distributed globally and presents a significant public health challenge. Diabetes effects on an individual of all ages and socioeconomic backgrounds, and its prevalence is increasing worldwide. Chronic hyperglycemia in diabetes is associated with prolonged damage, failure, and dysfunction of multiple organs, particularly the nerves, heart eyes, kidneys, and the vascular system [3]. Diabetes mellitus is a metabolic condition with multiple underlying causes, marked by chronic elevated blood glucose levels and disturbances in carbohydrate metabolism, involving disruptions in the metabolism of fats and proteins due to impairments in insulin release, insulin function, or together [4]. Type 2 diabetes mellitus (T2DM) is defined by insulin resistance, a state where the body's cells do not respond properly to insulin, and insufficient insulin release from beta cells of the pancreas. T2DM is among the predominant widespread metabolic disturbances globally, mainly resulting from two primary factors: reduced insulin secretion from pancreatic  $\beta$ -cells and the resistance of insulin-sensitive tissues to insulin [5]. Vitamin D, or cholecalciferol, is metabolized in the body to produce 25-hydroxyvitamin D [6]. it can be supplemented through sunlight exposure and dietary intake [4]. As an essential substance in the human body, inadequate Vitamin D3 levels are associated with various diseases, such as T2DM, tumor, autoimmune disorders, proteinuria, and high blood pressure [7]. There has been a rise in the prevalence

of T2DM observed in individuals with vitamin D shortage, together with impaired insulin production and insulin release in beta cells from animals deficient in vitamin D. Glucose tolerance improves when vitamin D levels are restored to normal.

Vitamin D deficiency or low level was linked to impairments glucose test (IGT) and T2DM in humans for many years [8]. These findings were validated in animal studies, where vitamin D shortage was found to inhibit pancreatic insulin secretion [9]. Several research has attributed a key function for vitamin D in managing the role of the endocrine pancreas, particularly the  $\beta$ -cells. In 2019, Wallace et al. carried out a dual-blind, randomness, dummy treatment - controlled trial, which a statement that vitamin D supplementation showed no impact on insulin hormone levels in individuals with prediabetes [10]. By Niroomand et al. [11] The study revealed that large - dosage vitamin D supplementation can enhance insulin responsiveness and reduce the venture of developing diabetes in individuals diagnosed with prediabetes and vitamin D deficiency. Several cross-sectional studies have investigated the relationship among vitamin D amount and the incidence of glucose intolerance in addition to type 2 diabetes. While the most of these studies have shown a negative relation among glucose intolerance and vitamin D levels, some did not find a significant association, as reviewed by Pittas et al. [2].

Analyzing glycated hemoglobin (HbA1c) in the blood offers an indication of an individual average typical blood glucose amount over the gone two to three months, which corresponds to the estimated lifespan of erythrocytes [12]. HbA1c testing is currently endorsed as a standard treatment for both diagnosing and managing diabetes, especially T2DM [13].

Ferritin is responsible for intracellular iron storage and is structurally consisting of 24 subunits, including both light chains and very weighty chains [14]. Serum ferritin is an acute-phase reactant and a measure of the body's iron reserves [15]. Chemically, iron is a transition metal that fast oxidizes and hence acts as an oxidant. Ferritin is an inflammatory marker as well as a measure of body iron [16]. Because of an increase in cellular secretion, ferritin levels rise intracellularly in large cell types and extracellularly in the plasma. Ferritin plays a vital function during the acute phase response by sequestering iron within the cavities of the ferritin protein shell [17]. As a consequence, under typical physiological conditions, free radicals are typically created in small amounts and are neutralized by endogenous antioxidant systems. This antioxidant system comprises enzymes like as superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as particles like as glutathione (GSH), bilirubin, uric acid, lipoic acid, transferrin, albumin, vitamins E and C, copper, carotenoids, and zinc.[18]. Antioxidants counteract free radicals through multiple mechanisms. Enzymes break down reactive oxygen species, while proteins such as transferrin bind to specific metals. that promote free radical production. Additionally, vitamins E and C act as scavengers, targeting and neutralizing free radical oxidation [19]. In diabetes and other disease states, this antioxidant defense mechanism is compromised, leading to ineffective elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This ineffectiveness plays a significant contribution to tissue damage in diabetic patients [20]. The higher levels of free radicals activate stress-signaling pathways and reduces both types of antioxidants, including enzymatic and non-enzymatic, negatively affecting the patient's quality of life and lifespan. Free radical ROS and RNS are implicated in a range of conditions, including diabetes and its complications [21].

## MATERIAL AND METHODS

### Subjects

The research was conducted over the course of one year (2023 to 2024). The research comprised of 50 T2DM patients 40 to 70 year-old and 50 normal subject as a control who were age and sex-matched.

### Exclusion Criteria

Patients with Human immunodeficiency virus (HIV) infection, as well as those with other long-term conditions, such as thyroid disorders, tuberculosis, coronary artery conditions, renal disease and complications, or additional issues of diabetes, were excluded from this study.

## Parameters and Sample Collection and Processing

Anticoagulant blood and serum were collected for blood tested with serum ferritin, vitamin D, total antioxidants status, and glycated hemoglobin. Patients' blood was drawn following an overnight fast (8-12 hours). Control participants were randomly chosen from the broader community according to specified criteria. Participants were briefed on the study, and participants who consented to participate were required to sign an informed consent form before their blood was collected. A questionnaire was employed to gather anthropometric data and general information from each participant, including medication use, physical activity, family history, and the length of diabetes duration, and other long-term illnesses. According to the guidelines of Helsinki Ethical Declaration (2013), the study was authorized by the Bioethics Committee in Scientific Research and the license number was BCSR2.

Blood collected after fasting samples (approximately 20 mL) were obtained from each individual using vacutainer tubes. If not assessed on the very same day, Serum samples were kept at  $-70^{\circ}\text{C}$  and analyzed within one month. These blood samples were utilized for measuring vitamin D, HbA1c, total antioxidants levels, and ferritin.

## Methods

Plasma HbA1c, vitamin D and ferritin were assessed using standard methods, using the instrument Fincare<sup>tm</sup> (FIA Meter Plus. No: FS-113). Serum total antioxidant activity levels were determined using a colorimetric technique.

## Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26 (IBM Corp, Armonk, NY, USA). Data are shown as mean  $\pm$  SE for factors with a natural distribution. The statistical importance of the variance among group means was evaluated using a t-test was applied for data with a natural division. For variables that did not keep track of a natural division the non-parametric Mann–Whitney U test was employed. Bivariate correlations between continuous variables were analyzed using Spearman rank correlation.

## RESULTS

### Demographic Charachtrization

The descriptive statistics of patients are illustrated in Table 1.

**TABLE 1.** The descriptive statistics of patients

Characteristics	Patients n=50
Age	n (%)
40-49 year	23 (46)
50-59 year	21 (42)
60-70 year	6 (12)
Gender	n (%)
Female	28 (56)
Male	22 (44)

### Main Results

The mean serum ferritin and total antioxidant levels were significantly inverse with ( $P=0.003$ ,  $P<0.001$ ). In a row in diabetic group in comparison with that of Controls (Table 2) also showed a revealed a positive correlation between among serum ferritin and total antioxidants.

There is a prominent decrease in serum levels of total antioxidants was noted in diabetic group patients in compare with that of healthy participants ( $p = 0.001$ ).

There were no notable variations HbA1c level between different groups ( $p=2.4$ ) and no significant differences vitamin D level among different groups ( $p=0.09$ ).

**TABLE 2.** Biostatistical analysis values for different parameters of patients with diabetes mellitus and control group

Parameters	Patients Mean $\pm$ SE	Healthy controls Mean $\pm$ SE	P-value
Vitamin D (ng/ml)	22.02 $\pm$ 1.37	17.61 $\pm$ 1.09	0.014*
Ferritin(ng/ml)	96.78 $\pm$ 9.00	66.37 $\pm$ 3.67	0.003**
HbA1c(mg/dL)	8.46 $\pm$ 0.22	5.71 $\pm$ 0.10	<0.001**
Total antioxidants mM	0.52 $\pm$ 0.43	1.72 $\pm$ 1.36	<0.001**
RBS (mg/dL)	213.98 $\pm$ 10.99	137.92 $\pm$ 2.39	<0.001**
Urea (mg/dL)	31.38 $\pm$ 3.95	30.76 $\pm$ 2.09	0.89
Creatinine (mg/dL)	1.28 $\pm$ 0.11	0.94 $\pm$ 0.03	0.005*
Uric acid (mg/dL)	5.88 $\pm$ 0.27	5.46 $\pm$ 0.15	0.172
TG (mg/dL)	201.48 $\pm$ 16.45	177.80 $\pm$ 5.89	0.18
Cholesterol (mg/dL)	183.72 $\pm$ 8.53	193.74 $\pm$ 5.76	0.333
LDL (mg/dL)	97.62 $\pm$ 7.07	113.86 $\pm$ 3.41	0.042*
VLDL (mg/dL)	38.29 $\pm$ 2.74	36.84 $\pm$ 2.26	0.683
HDL (mg/dL)	45.26 $\pm$ 1.21	52.52 $\pm$ 1.92	0.002**

**Notes:** details are offered as mean  $\pm$  SE and median (IQR), HbA1c= glycated hemoglobin, Vit D= vitamin D, the difference between the median and mean is statistically at \*p < 0.05 and \*\*p < 0.005 levels.

**TABLE 3.** Correlation analysis values for different parameters of patients with diabetes mellitus and control group

Characteristic	Vitamin D3		Ferritin		HbA1c		Total antioxidant	
	r	P	R	P	r	P	r	P
Vitamin D3	1							
Ferritin(ng/ml)	0.49	<0.001	1					
HbA1c(mg/dL)	0.34	0.015	0.197	0.170	1			
Total antioxidants mM	0.19	0.484	-0.42	0.012	0.12	0.671	1	
RBS (mg/dL)	0.008	0.954	0.229	0.110	0.134	0.353	-0.01	0.872
Urea (mg/dL)	-0.23	0.099	-0.063	0.662	-0.165	0.253	0.18	0.224
Creatinine (mg/dL)	-0.29	0.039	-0.162	0.262	-0.084	0.56	-0.21	0.194
Uric acid (mg/dL)	-0.32	0.02	-0.144	0.318	-0.188	0.190	-0.03	0.774
TG (mg/dL)	0.067	0.645	0.136	0.346	0.096	0.508	-0.09	0.513
Cholesterol (mg/dL)	0.079	0.586	0.127	0.380	0.239	0.095	0.13	0.389
LDL (mg/dL)	0.78	0.591	0.114	0.430	0.279	0.05	0.24	0.06
VLDL (mg/dL)	0.20	0.163	0.235	0.101	0.219	0.126	0.06	0.646
HDL (mg/dL)	-0.227	0.112	-0.31	0.030	-0.328	0.02	0.14	0.399

## DISCUSSION

The HbA1c is a very important indicator because reflect the cumulative impact glycemic disease history from the previous two to three months also has a strong relation with the chance of long-term diabetes problems [22]. Their differences and rises up of HbA1c with diabetic in our research was distinguished; Although HbA1c has been shown to have a stronger association with insulin susceptibility in healthy subjects with standard glucose forbearance [23]. Consequently, HbA1c is considered a dependable biomarker and an important indicator of insulin resistance, making it an excellent tool for testing individuals with diabetes and prediabetes [24]. HbA1c and serum ferritin levels may be having been benefit to predict insulin resistance and illness time duration [25]. Increasing blood iron as storage, as measured by serum ferritin, are assumed to have a role in the progression of T2DM [26]. In the present study saw highly increasing in the ferritin in the test group; which these phenomena belonged to iron accumulation in the liver may prevent insulin from acting on the liver [27].

Furthermore, in our research, we noted in obvious a reduced total antioxidant standing in diabetic instance ( $0.46 \pm 0.46$  mM) compared to healthy controls ( $1.69 \pm 1.34$  mM). This reduction in diabetic subjects may be ascribed to heightened oxidative stress, as indicated due to fat peroxidation. The decline in antioxidants send back struggle between antioxidants and oxidative stress to reduce oxidative damage. When the total antioxidant standings elevated and adequate to neutralize oxidative stress, malondialdehyde (MDA) levels—measured by the spectrophotometric assay of thiobarbituric acid reactive substances (TBARS)—remain within normal limits. Conversely, when antioxidant levels are low, MDA levels may rise [17].

Total antioxidant status provides a comprehensive measure of both exogenous and endogenous antioxidants, offering a complete picture of the antioxidant defense. This approach is more significant than assessing individual antioxidants because numerous antioxidants labor synergistically to battle oxidative harm create by without charge radicals. The academic work on antioxidant vitamins behave by Andhra and Suchitra et al. [28] showed a decrease in extracellular antioxidant levels in patients with T2DM, irrespective of complications. Mahmood, I. H. et al. reported a lower overall antioxidant levels in diabetic patients compared to those with nerve damage, as measured utilizing the Cayman kit. [29]. Multiple studies have shown reduced antioxidant levels and increased peroxidative standing in diabetic conditions. [30].

Vitamin D is a versatile hormone that influences various crucial biological roles, including immune system rule and metal ion metabolism. While its primary is to regulate calcium and phosphate balance and aid in bone mineralization, numerous extrskeletal functions of vitamin D have also been identified. [31].

In our study of an Asian inhabitants at high chance for T2DM, we found that participants with 25(OH)D insufficiency had a T2DM occurrence 3.4 times higher than those with enough amount. This connection persisted significant even after making adjustments for factors such as fatness, insulin opposition to, pancreatic  $\beta$ -cell purpose, and other familiar T2DM risk factors. To date, significant inverse relationships among serum 25(OH)D levels and T2DM or impaired glucose metabolism have been observed in cross-sectional studies [32]. Older individuals with low 25OHD levels may be less susceptible to health issues related to hypovitaminosis D compared to those who had low 25OHD levels and developed diabetes at a younger age [33]. It is important to highlight that none of the longitudinal studies found a notable correlation between baseline hypovitaminosis D and the incidence of diabetes after adjusting for potential confounders. Additionally, insulin resistance has been found to be associated with vitamin D insufficiency [2]. Vitamin D may straight effect insulin responsiveness by activating insulin receptor expression. These findings propose a strong bind among 25(OH)D levels and inflammatory processes, indicating a potential mechanism by which vitamin D could be included in the evolution of T2DM.

## CONCLUSION

The current study suggested the significant role of increased ferritin in pathogenesis of T2DM due to accumulation of ferritin in the liver prevents insulin from responding. In Iraqi diabetic patients, there is a strong link between circulating ferritin levels and total antioxidants, while vitamin D status could contribute to maintaining glucose homeostasis.

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